Pharmaceutical Research, V.

Exhibit A

Evaluation of Sustained/Controlled-Release Dosage Forms of 3-Hydroxy-3-methylglutaryl–Coenzyme A (HMG-CoA) Reductase Inhibitors in Dogs and Humans

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Seven sustained/controlled-release dosage forms were designed for gastrointestinal delivery of lovastatin or simvastatin, two potent HMG-CoA reductase inhibitors for the treatment of hypercholesterolemia. The *in vivo* performance of these formulations was evaluated in dogs and healthy volunteers in terms of the cholesterol lowering efficacy and/or systemic concentrations of HMG-CoA reductase inhibitors. Results from the present and previous studies suggest that, through the controlled release of HMG-CoA reductase inhibitors, sustained lower plasma concentrations of HMG-CoA reductase inhibitors may result in an equal or better therapeutic efficacy.

KEY WORDS: lovastatin; simvastatin; sustained-release dosage forms; 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors; dog; humans.

INTRODUCTION

Lovastatin and simvastatin are members of a new class of drugs used in the treatment of hypercholesterolemia. Being lactone prodrugs, they hydrolyze *in vivo* to their corresponding β-hydroxyacids which are potent inhibitors of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase and, thus, of de novo cholesterol synthesis (1,2). Recently, Bradford *et al.* (3) reported that lovastatin, 20 mg twice daily, produced a significantly greater reduction in low-density lipoprotein-cholesterol levels in patients than a 40-mg once daily dosage. Subsequently, McClelland *et al.* (4) have shown in dogs that systemic concentrations of HMG-CoA reductase inhibitors could be minimized and the efficacy of the inhibitor could be enhanced by oral administration of the tromethammonium salt of the β-hydroxyacid of simvastatin through a controlled-release dosage form. Also, Duggan *et al.* (5) and Vickers *et al.* (6) reported that lovastatin and simvastatin were more efficiently extracted by the liver, which is the target organ for both compounds, than their corresponding hydroxyacids with subsequent minimization of systemic burden. These findings (3–6) suggest that, compared to a conventional dosage form, a sustained/controlled-release dosage form of lovastatin or simvastatin might provide similar or better efficacy. Accordingly, the development of sustained/controlled-release dosage formulations was initiated. This report summarizes the results of several studies in dogs and/or healthy volunteers to compare these formulations with the conventional dosage form in terms of plasma profiles of HMG-CoA reductase inhibitors and cholesterol lowering efficacy.

MATERIALS AND METHODS

Dosage Forms

Lovastatin. The following sustained/controlled-release dosage forms containing 40 mg of lovastatin were tested and compared with the 40-mg conventional lovastatin tablet (CT) in dogs: 8- and 14-hr sustained-release matrix tablet formulations (SRT8 and SRT14) and 8- and 14-hr controlled-release systems (CRS8 and CRS14). SRT8 and SRT14 are swelling sustained-release matrix formulations (7), while the CRS formulations are coated tablets from which release of drug is controlled by the coat.

Simvastatin. Three controlled-release dosage forms based on controlled-porosity osmotic pump principles (8) containing 20 mg of simvastatin were tested and compared with the 20-mg conventional simvastatin tablet (CT) in dogs or humans. The controlled-release simvastatin dosage forms were formulated as 8-, 12-, and 14-hr systems (MODS8, MODS12, and MODS14).

Dog Study I

This was a seven-way, sequential, crossover study in beagle dogs with a washout period of at least 6 days between each treatment. Six male beagle dogs weighing 13.4–19.2 kg were housed individually and fasted for 24 hr, then allowed to eat 1 hr before the 0-time sample and dosing (80 mg). Blood samples (5 mL) were collected from each dog at the following times: conventional tablets—predose, 0.5, 1, 2, 3, 4, 7, 10, 13, and 16 hr postdose; and sustained/controlled-release formulations—predose, 1, 2, 4, 7, 10, 13, 16, 20, and 24 hr postdose. For the lovastatin SRT8 treatment, blood samples were obtained for only up to 10 hr postdose. Based on results obtained from a previous dog study (4), the last sampling time for each treatment was chosen such that the plasma concentration of total HMG-CoA reductase inhibitory activity at that time was expected to be close to the lower quantitation limit (5 ng eq/mL) of the assay method

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(9). The seven treatments were completed in the following order:

(1) lovastatin SRT14 (2 × 40 mg),
(2) lovastatin CT (2 × 40 mg),
(3) simvastatin CT (4 × 20 mg),
(4) lovastatin CRS14 (2 × 40 mg),
(5) simvastatin MODS12 (4 × 20 mg),
(6) lovastatin SRT8 (2 × 40 mg), and
(7) lovastatin CRS8 (2 × 40 mg).

Plasma was obtained from each blood sample and maintained frozen at −20°C until the time of analysis.

Dog Study II

Based on the results of Dog Study I, which are discussed later, certain of the above dosage forms were evaluated in Dog Study II. This was a six-group (n = 5 dogs/group) noncrossover study in beagle dogs to determine the extent of serum cholesterol reduction associated with oral administration of lovastatin in CT or CRS or with simvastatin in CT or MODS. The weight, gender, and age of these dogs were matched between each group. Dogs weighing 10.4–22.79 kg (mean ± SD = 15.1 ± 3.3 kg) were administered a daily dose (10 mg/kg of lovastatin in CT, CRS8, or CRS14 or 10 mg/kg of simvastatin in CT, MODS8, or MODS14) 1 hr after feeding for 28 days. The dosage forms were administered with 10 mL of water. Baseline serum cholesterol was determined by weekly blood sampling for at least 3 weeks prior to dosing. Serum samples were obtained weekly prior to dosing (at approximately 9:30 A.M.) for the determination of serum cholesterol levels during the dosing periods. The mean maximum decrease from baseline for each group was calculated from the single lowest individual serum cholesterol value during the dosing interval for each dog. A cholesterol esterase procedure standardized against the Abell–Kendal reference method (10) was used to determine the serum cholesterol levels.

Human Study

This was a single-dose, double-blind, three-period crossover study. After fasting overnight, nine healthy males between 19 and 28 years of age, weighing between 61 and 90 kg, were randomized to receive one of the three treatment sequences shown in Table I.

Each treatment period was separated by a washout period of at least 6 days. In each treatment period, blood samples were taken prior to dosing and at 1, 2, 4, 6, 8, 12, 14, 16 and 24 hr postdose. Plasma was obtained from each blood sample and maintained frozen at −20°C until the time of analysis.

Table I. The Three Treatment Sequences in the Human Study

Treatment	No. of subjects	Period 1	Period 2	Period 3
1	3	A	B	C
2	3	B	C	A
3	3	C	A	B

A = 20-mg simvastatin conventional tablet and placebo capsule of MODS8 and MODS14
B = 20-mg simvastatin in MODS8 capsule and placebo of conventional tablet
C = 20-mg simvastatin in MODS14 capsule and placebo of conventional tablet

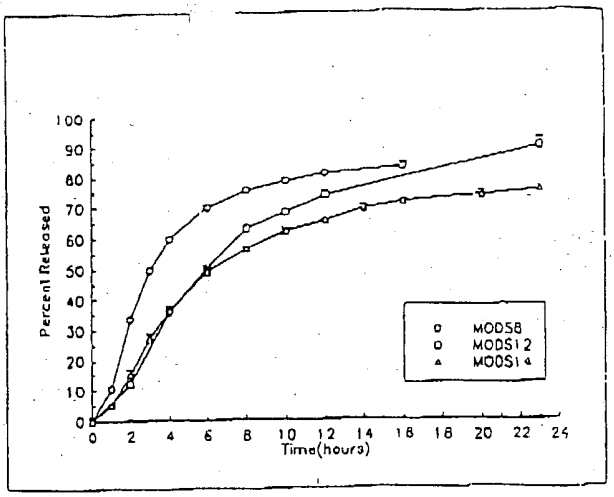

Fig. 1. Mean (±SD) *in vitro* release profiles from simvastatin MODS8 (n = 6), MODS12 (n = 3), and MODS14 (n = 6) devices.

Sample and Pharmacokinetic Analyses

Plasma samples from the human and dog studies were assayed for the concentration of total HMG-CoA reductase inhibitors using an enzymatic assay method (1,9). The observed maximum concentration of total HMG-CoA reductase inhibitors (C_{max}), the observed time of maximum inhibitory activity (T_{max}), and the area under the plasma profile of total HMG-CoA reductase inhibitors (AUC) were determined from these data. The AUC values were calculated using the trapezoidal rule from the time 0 to the last sampling time. In the dog and human studies, the AUC and C_{max} ratios were calculated from individual values following oral administration of a sustained/controlled-release formulation to those following oral administration of the conventional formulation.

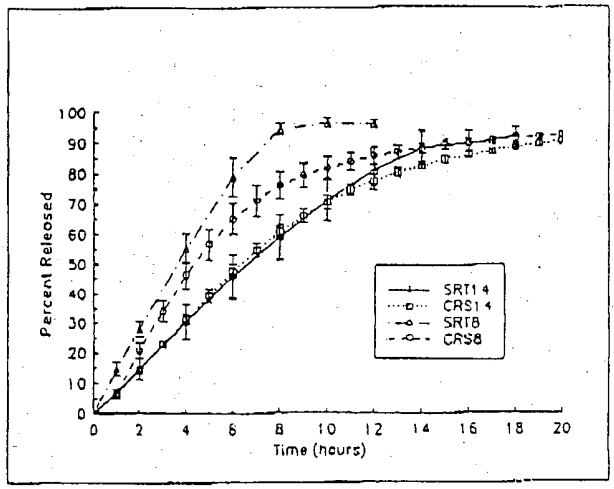

Fig. 2. Mean (±SD) *in vitro* release profiles from lovastatin SRT8 (n = 12), SRT14 (n = 12), CRS8 (n = 2), and CRS14 (n = 3) devices.

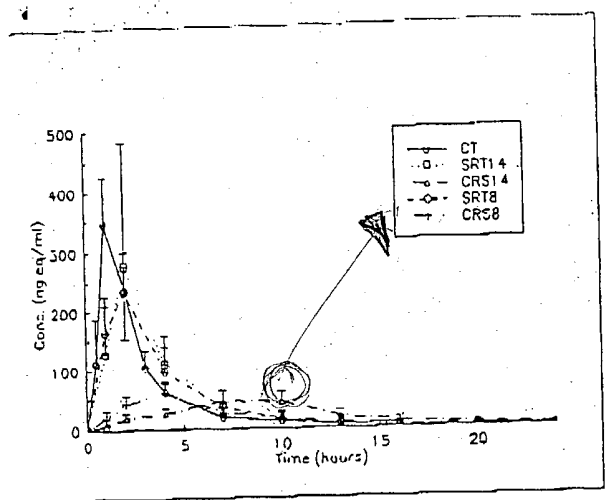

Fig. 3. Mean (SD; $n = 6$) plasma concentration–time profiles of total HMG-CoA reductase inhibitors in dogs receiving separately 80 mg lovastatin in five different formulations.

Comparisons of AUC, C_{max}, or cholesterol lowering between different treatments were made with ANOVA and differences were considered significant at $P < 0.05$. The *in vitro* release of lovastatin or simvastatin from sustained/controlled-release devices was followed by procedures similar to those been described previously (4).

RESULTS

As shown in Fig. 1, the MODS12 device released approximately 55% of its simvastatin content *in vitro* (pH 7.4; isotonic) with approximately zero-order kinetics over a 6-hr period, approximately 74% in 12 hr, and approximately 90% in 23 hr. Similarly, the MODS8 device released 75.8% in 8 hr and the MODS14 device released 69.7% in 14 hr (Fig. 1).

The SRT8 or SRT14 device released approximately 90% of the initial drug content in 8 or 14 hr with zero-order kinetics over a 6-hr period (Fig. 2). Similarly, the CRS8 and CRS14 devices released approximately 80% of the initial drug contents in the desired time frames with zero-order kinetics observed over a 4- to 5-hr period (Fig. 2).

It has been reported previously (5,6) that the dog is an appropriate paradigm for man for the study of certain qualitative aspects of lovastatin and simvastatin disposition. Thus, together with CRS8, CRS14, MODS12, and CT formulations, SRT8 and SRT14 formulations were administered to dogs. As shown in Fig. 3 and Table II, the mean C_{max} was approximately 35% lower ($P = 0.0486$ for SRT8 and $P = 0.1754$ for SRT14) and the mean AUC was equal to or 10% greater ($P > 0.05$) when dogs received SRT8 or SRT14 as compared to when they received CT. In the same comparison, T_{max} was only approximately 1 hr later for either SRT formulation. The mean AUC value for total inhibitors was approximately 50% lower ($P < 0.05$) in dogs receiving a single dose of 80-mg lovastatin in CRS8 or CRS14 formulations than when they received the CT formulation (Table II). Corresponding mean C_{max} values were approximately 85% lower ($P < 0.001$). On average, the T_{max} of total inhibitors occurred approximately 3–7 hr later in dogs receiving lovastatin in CRS8 and CRS14 formulations than with the CT formulation. The mean AUC value for total inhibitors (418 ng eq · hr/mL; Table II) was slightly lower in dogs receiving a single dose of 80-mg lovastatin in the CRS8 formulation than when they received the CRS14 formulation (487 ng eq · hr/mL). Conversely, the corresponding mean C_{max} value (Table II) appears to be higher. These differences, however, were not statistically significant ($P > 0.05$).

Similarly, the mean AUC value for total inhibitors is approximately 75% ($P < 0.005$) lower in dogs receiving a single dose of 80 mg of simvastatin from the MODS12 formulation compared to the CT formulation (Table III and Fig. 4), while the corresponding mean C_{max} value is approximately 90% lower ($P < 0.005$). On average, the T_{max} values of total inhibitors occurred approximately 4 hr later in dogs receiving simvastatin in the MODS12 formulation than when they received the CT formulation.

Because the SRT8 and SRT14 dosage forms showed little evidence of *in vivo* sustained-release functionality, they were dropped from further consideration. To investigate whether controlled delivery of HMG-CoA reductase inhibitors, with the attendant reduction in plasma profile of HMG-CoA reductase inhibitory activity, could maintain cholesterol lowering efficacy as compared to the CT formulation, the *in vivo* cholesterol lowering efficacy of the MODS and

Table II. Mean (±SD; $n = 6$) Pharmacokinetic Parameters of Total HMG-CoA Reductase Inhibitors in Dogs Receiving a Single Oral Dose of 80 mg Lovastatin in Five Different Formulations

Lovastatin formulation	AUC (ng eq · hr/mL)	C_{max} (ng eq/mL)	T_{max} (hr)	AUC ratio[a]	C_{max} ratio[a]
CT	901 ± 161	359 ± 76	1.0 ± 0.4	—	—
SRT8	888 ± 345 ($P > 0.05$)	237 ± 59 ($P = 0.0486$)	1.8 ± 0.4	0.94	0.66
SRT14	998 ± 462 ($P > 0.05$)	277 ± 206 ($P = 0.1754$)	2.3 ± 0.8	1.03	0.64
CRS8	418 ± 180* ($P < 0.05$)	59.7 ± 17.9** ($P < 0.001$)	4.0 ± 0.0	0.43	0.16
CRS14	487 ± 181 ($P < 0.05$)	48.0 ± 16.7 ($P < 0.001$)	7.5 ± 1.2	0.52	0.13

[a] Geometric mean of individual ratios.
* Compared with AUC of total inhibitors in dogs receiving CRS14, $P > 0.05$.
** Compared with C_{max} of total inhibitors in dogs receiving CRS14, $P > 0.05$.

Table III. Mean (±SD; n = 6) Pharmacokinetic Parameters of Total HMG-CoA Reductase Inhibitors in Dogs Receiving a Single Oral Dose of 80 mg Simvastatin in Two Different Formulations

Simvastatin formulation	AUC (ng eq · hr/mL)	C_{max} (ng eq/mL)	T_{max} (hr)	AUC ratio[a]	C_{max} ratio[a]
CT	1388 ± 573	660 ± 371	1.1 ± 1.0	—	—
MODS12	361 ± 215 ($P < 0.005$)	49.8 ± 24.2 ($P < 0.005$)	5.0 ± 1.7	0.23	0.07

[a] Geometric mean of individual ratios.

CRS dosage forms was compared to the CT formulation in dogs. As shown in Table IV, the dosing regimen of lovastatin in CRS formulations or simvastatin in MODS formulations was as effective at lowering cholesterol as either drug in the corresponding CT formulation at an equivalent total daily dose ($P > 0.05$). Interestingly, the coefficients of variation of cholesterol lowering response after multiple dosing (Table IV) were relatively low compared to that of AUC values after single doses in dogs (Tables II and III).

Given these results and the availability of animal safety data which allowed Phase I evaluation in humans, simvastatin MODS formulations with two different release rates and the CT formulation were administered to healthy males so that plasma profiles of HMG-CoA reductase inhibitors could be compared. The mean AUC value for total inhibitors was 54% lower ($P < 0.01$) in subjects receiving a single dose of 20-mg simvastatin in MODS14 than when they received the conventional formulation. The corresponding mean C_{max} value was 73% lower ($P < 0.001$; Table V). On average, the T_{max} value of total inhibitors occurred approximately 3–4 hr later in subjects receiving simvastatin in the MODS14 formulation than when they received the conventional formulation. The corresponding mean plasma concentration profiles of these inhibitors were also much prolonged (Fig. 5).

Similarly, the mean T_{max} value of total inhibitors occurred approximately 2–3 hr later in subjects receiving a single dose of 20-mg simvastatin in the MODS8 formulation than when they received the conventional formulation (Table V and Fig. 5). The corresponding mean C_{max} value was 57% lower ($P < 0.001$). The mean AUC value for total inhibitors was 18% lower in subjects receiving simvastatin in MODS8 formulation compared to those receiving the conventional formulation.

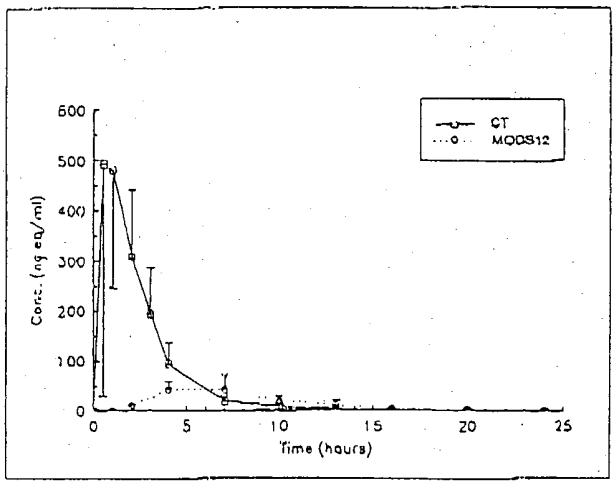

Fig. 4. Mean (SD; n = 6) plasma concentration–time profiles of total HMG-CoA reductase inhibitors in dogs receiving separately 80 mg simvastatin in two different formulations.

DISCUSSION

Although lovastatin was released *in vitro* from either SRT8 or SRT14 in a sustained-release fashion (Fig. 2), the plasma concentrations of total HMG-CoA reductase inhibitors in dogs receiving lovastatin in SRT8 or SRT14 were not much different from those in dogs receiving the same dose of lovastatin in CT (Table II and Fig. 3). These results indicate that SRT8 and SRT14 formulations for lovastatin do not perform *in vivo* as one would expect based on *in vitro* results, nor do they provide plasma profiles (Fig. 3) that are protracted. Thus, the *in vitro* release profile does not predict the *in vivo* performance of these sustained-release formulations of lovastatin in the dog.

On the other hand, the MODS formulations for simvastatin and the CRS formulations for lovastatin afforded plasma profiles (Tables II and III and Fig. 3 and 4) consistent with the *in vitro* release profiles (Figs. 1 and 2) and were as effective at lowering cholesterol as the CT formulation in dogs (Table IV).

When the MODS formulations were evaluated in humans, the mean plasma concentrations of total HMG-CoA reductase inhibitors in subjects receiving a single oral dose of 20 mg simvastatin in MODS14 were approximately 50% lower than those in subjects receiving the same dose of simvastatin in conventional tablet. Corresponding mean plasma concentration profiles of these inhibitors were also much prolonged (Fig. 5). These prolonged profiles, together with

Table IV. Mean (±SD; n = 5) Maximum Reduction of Serum Cholesterol in Dogs Receiving Lovastatin or Simvastatin (10 mg/kg) in Conventional or Sustained/Controlled-Release Dosage Forms for 28 Days

Formulation	Baseline cholesterol (mg/100 mL)	Maximum percentage decrease in serum cholesterol
Simvastatin CT	191 ± 25	21 ± 8.9
Simvastatin MODS8	206 ± 35	28 ± 8.8
Simvastatin MODS14	212 ± 38	23 ± 9.7
Lovastatin CT	150 ± 90	24 ± 18
Lovastatin CRS8	189 ± 33	13 ± 4.9
Lovastatin CRS14	193 ± 37	24 ± 7.6

Table V. Mean (±SD; $n = 9$) Pharmacokinetic Parameters and Ratios of AUC and C_{max} of Total HMG-CoA reductase Inhibitors in Healthy Subjects Receiving Separately a Single Dose of 20 mg Simvastatin in Three Different Formulations

Simvastatin formulation	AUC (ng eq · hr/mL)	C_{max} (ng eq/mL)	T_{max} (hr)	AUC ratio[a]	C_{max} ratio[a]
CT	61.9 ± 20.6	18.4 ± 7.3	1.7 ± 1.0	—	—
MODS8	53.9 ± 25.8 ($P > 0.05$)	7.8 ± 2.1 ($P < 0.001$)	4.2 ± 0.7	0.82	0.43
MODS14	36.7 ± 25.6 ($P < 0.01$)	5.2 ± 2.1 ($P < 0.001$)	4.7 ± 1.0	0.46	0.27

[a] Geometric mean of individual ratios.

the later T_{max} and significant reduction in both AUC and C_{max} of total HMG-CoA reductase inhibitors following administration of MODS14 compared to administration of CT, indicate that simvastatin was released *in vivo* in a controlled fashion. Like simvastatin in MODS14, but to a lesser degree, simvastatin in MODS8 was also released *in vivo* in a controlled fashion (Table V, Fig. 5). Thus, the degree of plasma profile alteration/prolongation for inhibitory activity appears to be affected in the manner expected by the release rate of simvastatin from MODS formulations in humans.

Judging from the results in dogs and humans described above, the CRS formulations may well function in the same fashion in humans. The performance of these lovastatin CRS formulations in humans and the ability of the MODS formulations for simvastatin to reduce cholesterol as effectively as equal or greater daily doses of the drug in the CT formulation are currently under evaluation.

By comparing the AUC and C_{max} ratios for CRS and MODS formulations in dogs (Tables II and III) to those in humans (Table V), it can be concluded that the dog may not be a good model for predicting the relative bioavailability of lovastatin or simvastatin in these formulations in humans.

In conclusion, results from the present and previous (3,4) studies indicate that the use of controlled-release HMG-CoA reductase inhibitors as a means of achieving sustained lower plasma concentrations with equal or better therapeutic efficacy merits further study in humans.

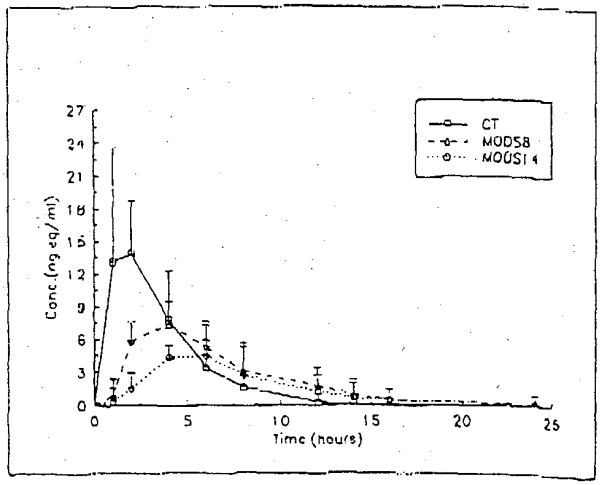

Fig. 5. Mean (SD; $n = 9$) plasma concentration–time profiles of total HMG-CoA reductase inhibitors in healthy volunteers receiving separately 20 mg simvastatin in three different formulations.

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